FUNCTION AND REGULATION OF CULLIN–RING UBIQUITIN LIGASES

Matthew D. Petroski and Raymond J. Deshaies

Abstract | Cullin–RING complexes comprise the largest known class of ubiquitin ligases. Owing to the great diversity of their substrate-receptor subunits, it is possible that there are hundreds of distinct cullin–RING ubiquitin ligases in eukaryotic cells, which establishes these enzymes as key mediators of post-translational protein regulation. In this review, we focus on the composition, regulation and function of cullin–RING ligases, and describe how these enzymes can be characterized by a set of general principles.

UBIQUITIN LIGASE (E3)
A protein or protein complex that facilitates the transfer of ubiquitin from the active-site cysteine of a ubiquitin-conjugating enzyme (E2) to a Lys residue of a substrate. Two main classes have been identified on the basis of the presence of either a HECT domain or a RING-like motif.

CULLIN
A family of proteins that is characterized by the presence of a distinct globular C-terminal domain (cullin-homology domain) and a series of N-terminal repeats of a five-helix bundle (cullin repeats).

Division of Biology and Howard Hughes Medical Institute, California Institute of Technology, 1200 East California Boulevard, Pasadena, California 91125, USA.
e-mails: petroski@caltech.edu; deshaies@caltech.edu
doi:10.1038/nrm1547

Multisubunit UBIQUITIN LIGASES (E3s) that are assembled on a CULLIN scaffold were first reported seven years ago[1,2]. The discovery of the archetypical cullin–RING ubiquitin ligase — SCF^{Cdc4} — benefited from a strong foundation of genetic studies on cell division in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*. The model established by SCF can now be extended to a superfamily of CULLIN–RING LIGASES (CRLS) that are found throughout eukaryotes. Together, these enzymes regulate a dazzling array of cellular and organismal processes — from glucose sensing and DNA replication to limb patterning and circadian rhythms.

Although there is a great diversity of CRLs in terms of their composition and function, we propose that these enzymes can be characterized by a set of general principles that will apply to most members of the superfamily. In this review, we summarize what has been learned about SCF and other CRLs over the past seven years and, from this, we extract the key features that typify these enzymes. We also highlight the murky areas in which our understanding remains far from clear.

Cullin–RING ligases are modular

The cullin family. Human cells express seven different cullins (CUL1, 2, 3, 4A, 4B, 5 and 7) that each nucleate a multisubunit ubiquitin ligase (FIG. 1). In addition, at least two other proteins (the APC2 subunit of the ANAPHASE-PROMOTING COMPLEX/CYCLOSOME (APC/C) and the p53 cytoplasmic anchor protein PARC) contain a 'cullin-homology domain'[3–5]. Although APC2 and PARC have ubiquitin-ligase activity, they are clearly distinct from the other cullins and are not considered here. The archetypal CRLs, which contain CUL1, are named SCF ubiquitin ligases, whereas the other CRLs have distinct subunit compositions and have been referred to by various names (TABLE 1).

Cullin–RING ligases have an extended, rigid architecture. Much of what is known and inferred about the architecture of CRLs comes from protein–protein interaction studies and sequence comparisons that have been interpreted in light of three-dimensional (3D) X-ray crystal structures (for a summary of the solved structures for CRL complexes and related proteins, see online supplementary information S1 (table)). CUL1 and presumably all other cullins have a curved, yet rigid, N-terminal stalk that is comprised of three repeats of a five-helix bundle (cullin repeat (CR)1–3) and is linked to a C-terminal globular domain[6] (FIG. 2). The SKP1 adaptor binds to the N-terminal CR1 region, whereas the zinc-binding RING-H2-DOMAIN protein — which is known as either ROC1, RBX1 or HRT1 (REFS 7–10; and is referred to here as the 'RING' subunit) — binds 100 Å away from SKP1 and interdigitates itself with the C-terminal globular domain. SKP1 recruits substrate receptors and the RING subunit recruits the ubiquitin-conjugating enzyme (E2) to form the active ligase complex. The rigidity of the N-terminal stalk of CUL1 might juxtapose the E2 and the substrate to favour ubiquitin transfer, because a mutation that

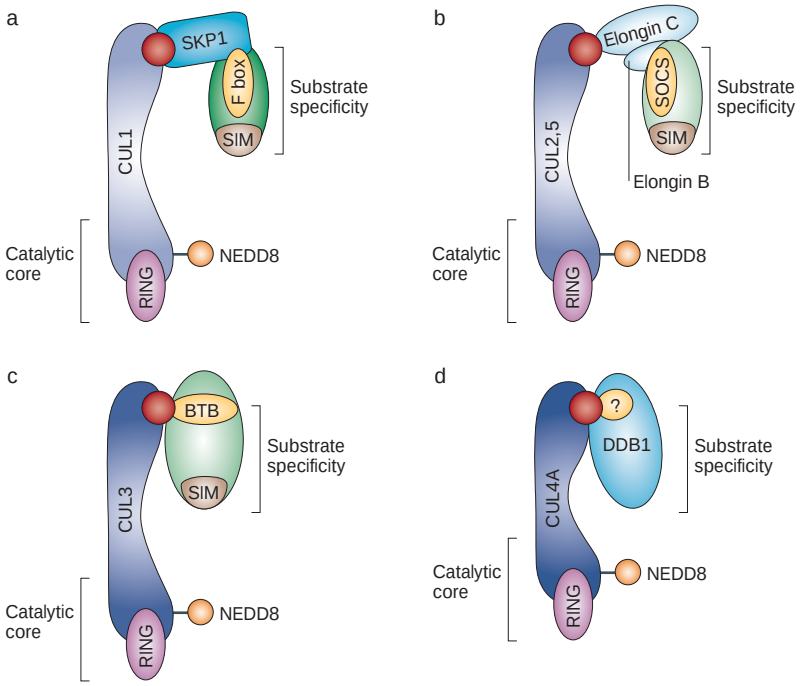

Figure 1 | **The modularity of cullin–RING ligases.** The common catalytic core of cullin–RING ligases (CRLs), which consists of a RING protein and a cullin-family member, defines this modular class of ubiquitin ligase. Structural analysis of cullin-1 (CUL1) showed that it has an elongated shape, with the RING protein bound to the C-terminal cullin-homology domain, near to a conserved Lys residue that is conjugated to NEDD8. By analogy to the structure of the RING-H2 ubiquitin ligase CBL, the RING subunit of CRL enzymes is thought to function as the docking site for ubiquitin-conjugating enzymes (E2s). Although the N-terminal region is thought to be similar in structure between family members, a segment of it is variable in sequence (red), and cullins use this region to interact with their own specific adaptor proteins. The motifs that link substrate receptors to their cognate adaptors are shown in yellow, although for CUL3 ligases, the adaptor and substrate-receptor functions are merged in a single 'Broad complex, Tramtrack, Bric-a-brac' (BTB)-domain-containing polypeptide. In addition to an adaptor-binding motif, substrate receptors contain a second protein–protein interaction motif that binds to the targeted substrate (substrate interaction motif (SIM), which is shown in brown). **a** | CUL1 CRLs, which are known commonly as SKP1, CUL1, F-box (SCF) proteins, recruit substrates through the adaptor protein SKP1 and an F-box-protein substrate receptor. **b** | CUL2 and CUL5 CRLs recruit substrates through an elongin-BC adaptor and a suppressor of cytokine signalling/elongin BC (SOCS/BC)-box-protein substrate receptor (labelled SOCS in the figure). **c** | CUL3 CRLs recruit substrates through BTB-domain-containing substrate-receptor proteins. **d** | CUL4A CRLs might recruit substrates through the adaptor protein DNA-damage-binding protein-1 (DDB1), which interacts with CUL4A using an unknown motif (highlighted by '?'), and through putative substrate-receptor complexes such as DET1–COP1.

increases the flexibility of the stalk destroyed SCF^{SKP2} activity *in vitro*[6].

A large body of evidence indicates that CRLs probably share a similar modular architecture (FIG. 1). Whereas the N-terminal domain of CUL1 docks substrates to SCF by binding the SKP1 adaptor, the N termini of CUL2 and CUL3 recruit substrates by binding to structurally similar elongin C and 'Broad complex, Tramtrack, Bric-a-brac' (BTB) DOMAINS, respectively. The more highly conserved C-terminal domains of cullins bind to the RING subunit ROC1, except for CUL5, which seems to prefer ROC2 (REF. 8). However, despite the overall similarities, there are variations on the theme. For example, CUL4A lacks most of the first cullin repeat[6], which indicates that its interaction with adaptor proteins and the relative spacing between the bound substrate and E2 might differ.

SCF
A multisubunit ubiquitin ligase (E3). It consists of SKP1, CUL1 and an F-box protein that confers substrate specificity, as well as a RING protein that is also known as HRT1, RBX1 or ROC1.

CULLIN–RING LIGASES
(CRLs). A superfamily of ubiquitin ligases that is characterized by an enzymatic core that contains a cullin-family member and a RING protein. The core is linked to specific substrates by adaptor proteins (or domains) and various receptor subunits.

Substrate receptors for cullin–RING ligases

Substrates are recruited to the N-terminal domains of cullins by a receptor module that comprises cullin- and substrate-binding domains. These domains can reside in the same or distinct polypeptide chains. Large families of these receptor modules greatly diversify the range of substrates that can be ubiquitylated by each CRL.

Substrate receptors are recruited by an adaptor. In the canonical example of CUL1, substrate receptors are recruited by the adaptor protein SKP1. SKP1 is divided into two domains: an N-terminal segment that binds CUL1 and a C-terminal region that binds the F-BOX MOTIF of substrate receptors[6,11] (FIG. 1a). The N-terminal segment of CUL1 that contacts SKP1 is highly conserved among CUL1 orthologues from different species, but not among other cullins, which reveals a structural basis for how each cullin specifically recruits a different set of substrates[6].

SKP1 interacts selectively with CUL1 and CUL7 (REFS 12,13). However, the adaptor proteins that are used by other cullins show considerable structural homology to SKP1. The cullin-binding region of elongin C, which binds CUL2 and CUL5 (REF. 14) (FIG. 1b), shares with SKP1 a fold that was first observed for the BTB domain[11]. This indicated that BTB-domain proteins might be adaptors for a CRL, and this has now been shown to be the case for several BTB-domain proteins, which bind specifically to CUL3 (REFS 15–18) (FIG. 1c).

The most poorly understood adaptor protein, DNA-damage-binding protein-1 (DDB1), is not related to SKP1, elongin C or BTB domains, so it is unclear how it links substrate receptors to CUL4A (REFS 19–21). As DDB1 is a much larger protein than SKP1 and elongin C, it might somehow compensate for the shorter length of the N-terminal stalk region of CUL4A (FIG. 1d).

Cullin–RING ligases assemble with numerous receptors. A key feature of CRLs is that each cullin can assemble with numerous substrate receptors to form ubiquitin ligases that share a common catalytic core yet recruit different substrates. These receptors typically contain an N-terminal domain that binds an adaptor and a C-terminal region that binds substrate. Two distinct adaptor-binding domains are known: F-boxes[22] bind SKP1, which, in turn, binds CUL1 and CUL7 (although only one F-box protein, FBW8/FBX29, has been shown to assemble with CUL7); whereas suppressor of cytokine signalling/elongin-BC (SOCS/BC) boxes[23] bind elongin BC, which, in turn, binds CUL2 and CUL5 (FIG. 1a,b). For CUL3-based ligases, the adaptor and substrate-receptor functions are merged into a single polypeptide that binds CUL3 directly through an N-terminal BTB domain and the substrate through a C-terminal domain[15–18] (FIG. 1c). Substrate receptors that are recruited to DDB1–CUL4A do not share an obvious motif[21] (FIG. 1d).

The number of F-box, BTB and SOCS/BC-box proteins, and therefore the potential number of CRLs, is staggering (TABLE 2). Whereas *S. cerevisiae* might have a minimum of 11 different complexes, humans might

Table 1 | The cullin–RING-ligase family

Cullin	Subunit organization	Names
CUL1	F-box protein/SKP1/CUL1/RING	SCF, CDL1
CUL2	SOCS/BC-box protein/elongin BC/CUL2/RING	VBC, CBC, ECS, SCF2, CDL2
CUL3	BTB-domain protein/CUL3/RING	BCR3, SCF3, CDL3
CUL4A	Receptor/DDB1/CUL4A/RING	VDC, DCX, SCF4, CDL4
CUL4B	?/CUL4B/RING	None
CUL5	SOCS/BC-box protein/elongin BC/CUL5/RING	SCF5, CDL5
CUL7	FBX29/SKP1/CUL7/RING	SCF7, CDL7

BCR, 'BTB protein, CUL3, RING'; BTB, 'Broad complex, Tramtrack, Bric-a-brac'; CBC, 'cullin, elongin BC'; CDL, cullin-dependent ligase; CUL, cullin; DDB1, DNA-damage-binding protein-1; DCX, 'DDB1, CUL4A, X (for unknown) box'; ECS, 'elongin BC, CUL2, SOCS/BC box'; FBX29, F-box protein-29; SCF, 'SKP1, CUL1, F-box protein'; SCF2–5 and SCF7, SCF-like complexes-2–5 and -7; SOCS/BC, suppressor of cytokine signalling/elongin BC; VBC, 'von Hippel–Lindau (VHL), elongin BC'; VDC, V-dependent complex or virus-degradation complex or 'V–DDB1–CUL4A'.

have hundreds. In the face of this rather startling number, a key question is whether all (or most) of these proteins indeed function as receptor subunits for cullins. In truth, the answer is not known. Most of the F-box proteins that are encoded in the *S. cerevisiae* genome bind Skp1 *in vitro*[24] and there is strong evidence that at least four of them comprise ubiquitin ligases that specify the ubiquitylation of physiological substrates[1,2,10,25–27]. However, at least two *S. cerevisiae* F-box proteins[28,29] do not assemble into SCF complexes[30]. The 3D structure of SCF^{SKP2} (FIG. 2) revealed specific contacts between CUL1 and the F-box of SKP2, so the sequence of the F-box can influence the assembly of F-box–SKP1–CUL1 complexes[6]. It seems reasonable to anticipate that the same caveat might apply to SOCS/BC-box and BTB proteins, especially given that the BTB domain seems to have other functions (for a review, see REF. 31).

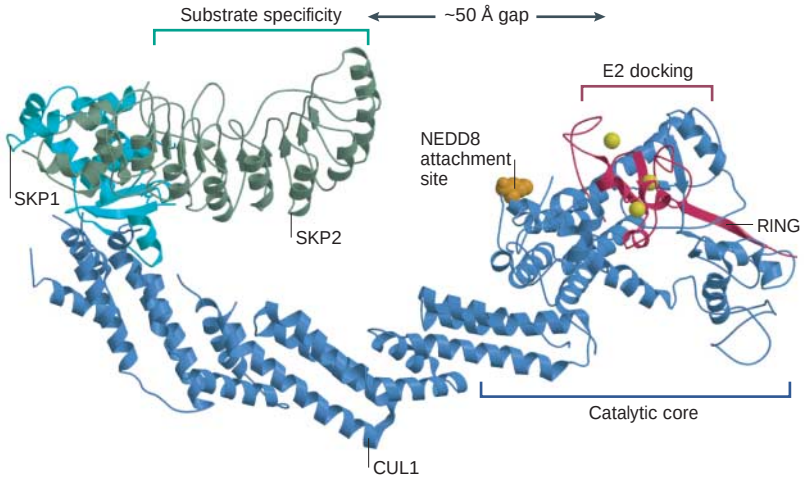

Figure 2 | **The structure of SCF^{SKP2}.** The crystal structure of SCF^{SKP2} highlights the extended modular nature of cullin–RING ligases[6] (Protein Data Bank file 1LDK). The F-box portion and the substrate-binding region of the substrate receptor SKP2 are shown — the former binds to the adaptor protein SKP1 and the latter extends towards the ubiquitin-conjugating enzyme (E2), which is bound to the E2-docking site on the RING protein. The substrate-specificity module of SCF is separated from the E2-docking site by a series of three cullin repeats that form the curved N-terminal stalk of cullin-1 (CUL1). The distance between a bound substrate and the active-site cysteine of an E2 that is docked on the RING protein has been estimated by computational methods to be ~50 Å. Yellow spheres represent zinc molecules, and the NEDD8 attachment site is highlighted. SCF, SKP1, CUL1, F-box protein.

Is there any simple rule that will allow us to predict whether F-box, SOCS/BC-box and BTB proteins assemble into a CRL? These proteins can be segregated into two categories — those that contain a second known protein–protein interaction domain and those that do not (TABLE 2). These domains include WD40 repeats, leucine-rich repeats, Kelch domains, CASH domains and zinc fingers. Of the four *S. cerevisiae* F-box proteins for which there is good evidence that they assemble into ligase complexes, all have conventional protein–protein interaction motifs that are situated C-terminal of the F-box. By contrast, both Ctf13 and Rcy1 — which do not form SCF complexes — lack such domains[29,32].

Receptors can form oligomers and are often unstable. Although we have described CRLs as comprising a substrate-binding polypeptide that is linked to the cullin through an adaptor protein, there are variations on this theme. Some receptor proteins, including the BTB-domain protein MEL-26 (REF. 16) and the F-box-proteins β-TrCP1/β-TrCP2 and Pop1/Pop2, can homo- or hetero-oligomerize, and this might expand the repertoire of substrates through a combinatorial mechanism[16,33,34]. The ability of some F-box proteins to oligomerize might be exploited to create dominant-negative mutants[35]. However, $SCF^{\beta\text{-TrCP}}$ that consists of a single monomer of β-TrCP can efficiently ubiquitylate a peptide substrate *in vitro*[36], so oligomerization is not required for E3 activity. The structure of receptor oligomers is not known because regions that are N terminal to the F-box that might mediate assembly were deleted from the F-box proteins for which structures have been solved[11,36,37]. Although the relevance of oligomerization remains unclear, there is excellent evidence that the function of F-box proteins can be promoted by unrelated binding partners. For example, the substrate-binding activity of the F-box-protein SKP2 is stimulated on assembly with CKS1 (REFS 38,39). A second example is the heterodimeric substrate-receptor complex DET1–COP1 that recruits the substrate Jun to DDB1–CUL4A (REF. 21).

The substrate receptors of CRLs are sometimes unstable. This is most evident in *S. cerevisiae*, in which F-box proteins are degraded with a half-life of 5–30 minutes after assembly with Skp1 (REFS 40,41), but has also been reported for MEL-26 in *C. elegans*[16]. By contrast, some mammalian substrate receptors are stable. A particularly striking example of this is the SOCS/BC-box protein SOCS1 (REFS 23,42), which is stabilized by assembly with elongin B and C (REF. 23). Although the destabilization of substrate receptors in *S. cerevisiae* was proposed to occur primarily by 'AUTOUBIQUITYLATION', this simple idea might not apply to mammalian counterparts. For example, the F-box-proteins TOME-1, SKP2 and EMI1 are turned over by regulated pathways that require APC/C (for TOME-1 and SKP2) or the F-box-protein β-TrCP (for EMI1)[43–47]. Nevertheless, autoubiquitylation — which is possibly regulated by the binding of substrate[48] — might be important in setting the steady-state level of some receptors[49].

Yet another variation on the theme of F-box-protein instability is provided by *S. cerevisiae* Ctf13. The F-box

Table 2 | CRL substrate-receptor subunits in various model organisms*

Protein–protein interaction motifs	Budding yeast	Fission yeast	Worm	Fly	Plant	Mouse	Human
F box	8	9	203	23	469	103	109
WD40	5	5	3	1	3	15	22
LRR	2	2	66	15	347	65	61
Kelch				1	41	2	2
Cyclin C						6	3
CASH						1	2
Zinc finger			1	2		2	2
None/other	1	2	133	4	78	12	17
BTB	3	4	213	250	104	375	439
Ankyrin	1	2	1	6	7	12	9
MATH			51	2	12	16	2
Kelch			10	21		85	125
Armadillo				1	5		
GTPase				2		11	6
Zinc finger				37	9	15	15
None/other	2	2	151	181	71	236	282
SOCS/BC box			2	9		45	51
Ankyrin			2			19	13
SH2				5		14	20
WD40						6	8
GTPase				3		2	6
None/other				1		4	4
Total	11	13	418	282	573	523	599

*This table shows the estimated number of putative substrate-receptor subunits for the various known cullin–RING ligases (CRLs) on the basis of data from the Pfam and SMART databases (see the online links box). Receptor subunits are first categorized by the motif that they use to interact with the CRL core, and are then further categorized by known secondary protein–protein interaction motifs. It should be noted that these numbers are estimates only and actual numbers might be considerably different. For example, there is a minimum of 19 different F-box proteins in yeast[30], at least 13 of which have been shown to bind Skp1 (REF. 24), but due to high variance in the F-box motif, not all are identified by the algorithms used by the Pfam and SMART databases. By contrast, an analysis of F-box proteins in human cells indicates that only ~70 exist and that the 109 predicted by Pfam and/or SMART is an overestimate that results from counting the same protein more than once[140] (see also 'HGNC Gene Family Nomenclature: F-Box gene family' in the online links box). The model organisms mentioned here are: budding yeast, *Saccharomyces cerevisiae*; fission yeast, *Schizosaccharomyces pombe*; worm, *Caenorhabditis elegans*; fly, *Drosophila melanogaster*; plant, *Arabidopsis thaliana*; mouse, *Mus musculus*; and human, *Homo sapiens*. BTB, 'Broad complex, Tramtrack, Bric-a-brac'; CASH, carbohydrate binding and sugar hydrolysis; LRR, leucine-rich repeat; MATH, meprin and TRAF-homology domain; SH2, Src-homology domain-2; SOCS/BC, suppressor of cytokine signalling/elongin BC; WD40, tryptophan and aspartate repeats.

of Ctf13 confers rapid turnover when it is fused to a normally stable protein[32], which indicates that the F-box itself might function as an internal DEGRON, similar to the destruction box in cyclin B. It is an open question whether Ctf13 or a 'canonical' receptor like Cdc4 is a more representative model for thinking about the large number of 'orphan' F-box proteins in *Arabidopsis thaliana* and *C. elegans*.

Regulating function without promoting degradation. Although most target proteins that bind to CRL substrate receptors are ubiquitylated as a prelude to degradation, proteolysis is not the only outcome. For example, ubiquitylation of the transcription factor Met4 by SCF^{Met30} inhibits its ability to activate transcription[50]. In a rich medium that contains high levels of methionine, SCF^{Met30} assembles a LYS48-LINKED POLYUBIQUITIN CHAIN on Lys163 of Met4 (REF. 51). Although ubiquitylated Met4 is not degraded, it no longer transactivates the expression of methionine-biosynthesis genes, but instead promotes the expression of genes that are involved in *S*-adenosylmethionine biosynthesis[52]. So, a stable Lys48-linked polyubiquitin chain reprogrammes the activity of Met4 by a mechanism that does not involve proteolysis.

Other atypical functions of CRLs include the processing of the *Drosophila melanogaster* Cubitus interruptus (Ci) protein and the human nuclear factor (NF)-κB precursor p100. Ci is processed from a 155-kDa transcriptional activator (Ci_{155}) to a 75-kDa transcriptional repressor (Ci_{75}). Genetic analysis indicates that sequential phosphorylation of Ci_{155} by three distinct protein kinases activates its ubiquitylation by SCF^{Slimb}, which leads to the PROTEASOME-mediated formation of Ci_{75} (REF. 53). Similarly, phosphorylation of p100 by inhibitor of NF-κB (IκB) kinase (IKK) activates $SCF^{β\text{-}TrCP}$-dependent ubiquitylation, which results in its proteasome-dependent cleavage to form the mature p52 subunit of NF-κB[54].

Most recently, it has been claimed that SCF^{SKP2} promotes the activation of genes that are regulated by the transcription factor Myc[55,56]. It was proposed that in the brief interval of time between its ubiquitylation and its destruction, Myc has an enhanced ability to promote transcription. Evidence that is consistent with this hypothesis is available for the synthetic transcription factor GAL4–VP16. SCF^{Met30} promotes both the ubiquitylation and transactivation ability of this transcription factor in *S. cerevisiae*[57]. Furthermore, the role of SCF^{Met30} in transactivation can be bypassed by expressing GAL4–VP16 that is fused to ubiquitin.

Substrate receptors have many targets and functions. Although it remains unclear how many of the human F-box, SOCS/BC-box and BTB-domain proteins assemble with cullins to form ubiquitin ligases, a survey of the available literature indicates that more than 50 different CRLs assemble in human cells (TABLE 2; see also online supplementary information S2 (table)). In addition, it is clear that these ligases help to regulate an awe-inspiring range of biological processes (FIG. 3). As a further testament to the physiological importance of the CRL pathways, receptors for these ligases are exploited by viral and bacterial pathogens to subvert normal cellular processes (see online supplementary information S3 (table)). For further reading on the physiological functions of CRLs and how their dysfunction contributes to disease, see REFS 58–61.

Post-translational substrate targeting

In almost every case that has been examined, substrates are targeted to CRLs by covalent modifications. Phosphorylation targets numerous substrates to SCF ligases, including the cyclin-dependent kinase (CDK) inhibitor Sic1 to SCF^{Cdc4} in *S. cerevisiae*, cyclin E to SCF^{CDC4} in humans, the CDK inhibitor p27 to SCF^{SKP2} in humans, and the G1-cyclin Cln2 to SCF^{Grr1} in *S. cerevisiae* (REFS 62–66). However, although phosphorylation

ANAPHASE-PROMOTING COMPLEX/CYCLOSOME (APC/C). A multisubunit ubiquitin ligase that contains a RING subunit (APC11) and a distant cullin homologue (APC2). It has a key role in regulating the eukaryotic cell cycle.

seems to be the predominant signal, core oligosaccharides that have a high mannose content are recognized by SCF$^{FBX2/FBS1}$ in humans (REF. 67), and a hydroxyproline epitope on hypoxia-inducible factor-1α (HIF1α) is recognized by the CUL2–elongin-BC–VHL (von-Hippel–Lindau) complex[68,69]. There is also at least one case in which a post-translational modification does not seem to be involved[16–18]. However, the general requirement that substrates must be modified before they can bind CRLs has at least four consequences, which are described in detail in the following paragraphs.

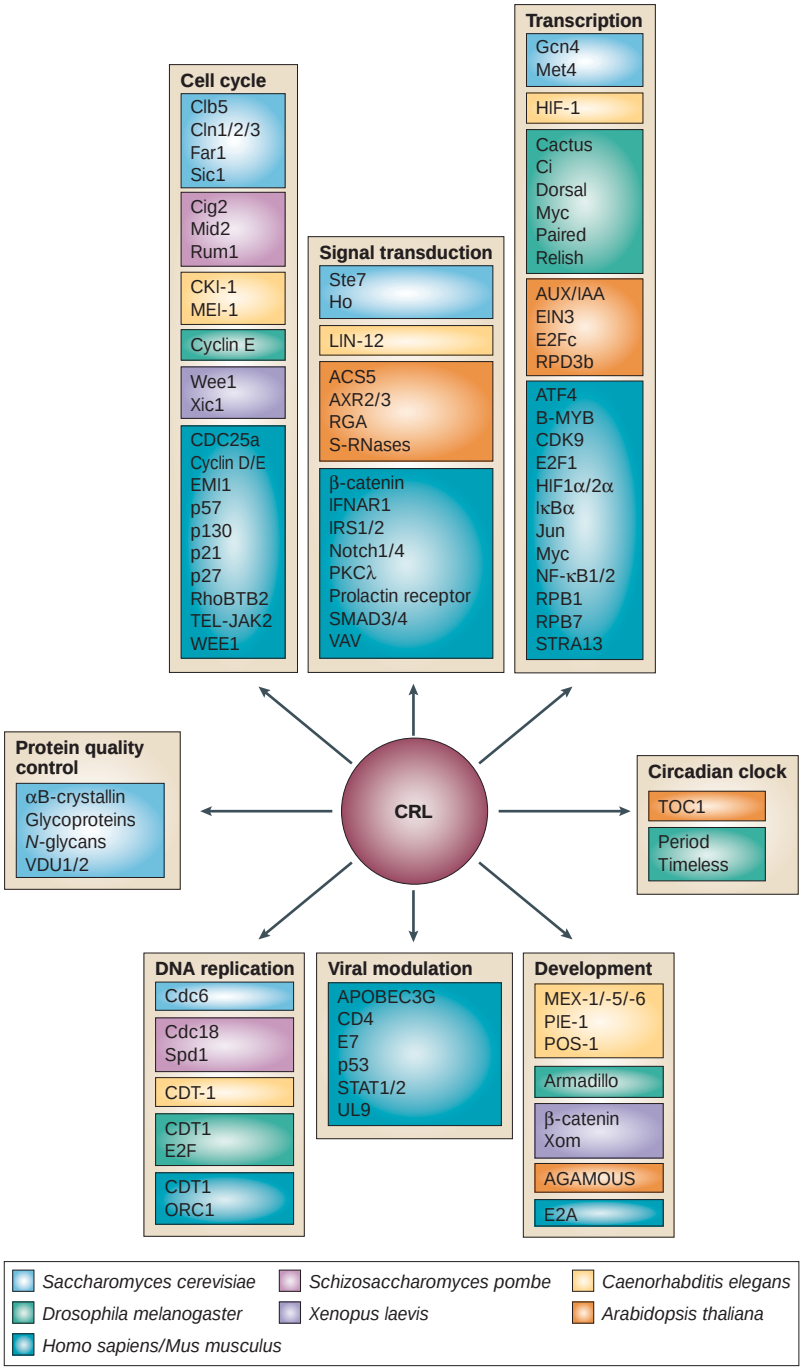

Figure 3 | **Role of cullin–RING ligases in diverse cellular processes.** Cullin–RING ligases (CRLs) have been implicated in a multitude of processes in numerous model organisms. Through the assembly of their enzymatic core with different substrate-recognition components, CRLs can promote the ubiquitylation of a wide array of targets. The diversity of proteins that can be targeted is further increased by post-translational modification, which is often required for substrates to be recognized by CRLs. This figure shows the cellular processes that are known to be regulated by CRLs, as well as some of the known substrates in various model organisms. For further information on the known CRL substrates, the receptors implicated in their targeting and the relevant references, see the online supplementary information S2 (table).

Covalent modifications expand the substrate repertoire. In *S. cerevisiae*, SCF^{Cdc4} regulates the cell cycle by promoting Sic1 turnover[70]. Sic1 binds and inhibits S-phase cyclin–CDK complexes, thereby blocking DNA replication. Late in G1, Sic1 is phosphorylated by G1 cyclin–CDK[62], which allows it to bind SCF^{Cdc4} and be ubiquitylated[1,2]. Ubiquitylated Sic1 is subsequently degraded by the proteasome, which yields active S-phase cyclin–CDK and results in entry into S phase[62,71]. Remarkably, SCF^{Cdc4} also regulates the expression of genes that are promoted by the transcription factor Gcn4 (a yeast activator that is involved in amino-acid and purine biosynthesis; REF. 72). Ubiquitylation of Gcn4 by SCF^{Cdc4} and its subsequent degradation requires the phosphorylation of Gcn4 by either the Pcl1/2–Pho85 or Srb11–Srb10 cyclin–CDK complexes[72,73]. Pcl1/2–Pho85 activity is reduced on amino-acid starvation, which enables Gcn4 to accumulate[74]. So, a single ubiquitin-ligase complex regulates distinct processes in response to distinct signals (FIG. 4a). The same is true for SCF$^{β\text{-}TrCP}$ in humans, which ubiquitylates β-catenin in response to glycogen synthase kinase-3 (GSK3) activity, and IκB in response to IKK activity[48].

Selective ubiquitylation of a specific substrate pool. CRLs often do not target the entire pool of a particular substrate (FIG. 4b). For example, ubiquitylation of the CDK inhibitor Xic1 by SCF in *Xenopus laevis* egg extracts depends on the replication-origin-licensing factor Cdc6, the origin-recognition complex and active cyclin-E–Cdk2. This indicates that the turnover of Xic1 is spatially constrained to sites at which DNA replication is initiated[75]. Another example is p27. Whereas the ubiquitylation of p27 by SCF^{SKP2} requires both the phosphorylation of p27 and its assembly into a trimeric complex with cyclin–CDK[76], not all cyclin–CDK–p27 complexes are substrates for SCF^{SKP2} (REF. 77). The remarkable specificity of CRLs for specific subsets of substrate molecules has enormous implications for the study of regulated protein turnover.

Generation of 'AND'/'OR' logic gates. The degradation of CRL substrates can be linked to signalling pathways by logic gates that are reminiscent of those used in computation (FIG. 4c). An example of an 'AND' gate is provided by cyclin E in humans. Cyclin E must be phosphorylated on both GSK3 'AND' CDK sites to be targeted efficiently to SCF^{CDC4} (REF. 78). A similar principle controls the ubiquitylation of Ci by SCF^{Slimb} in *D. melanogaster* (REF. 53). Conversely, *S. cerevisiae* Gcn4 provides an example of an 'OR' gate, in that Gcn4 can be targeted for SCF^{Cdc4}-mediated ubiquitylation and degradation by either the Srb11–Srb10 'OR' the Pcl1/2–Pho85 cyclin–CDK complex[72,73].

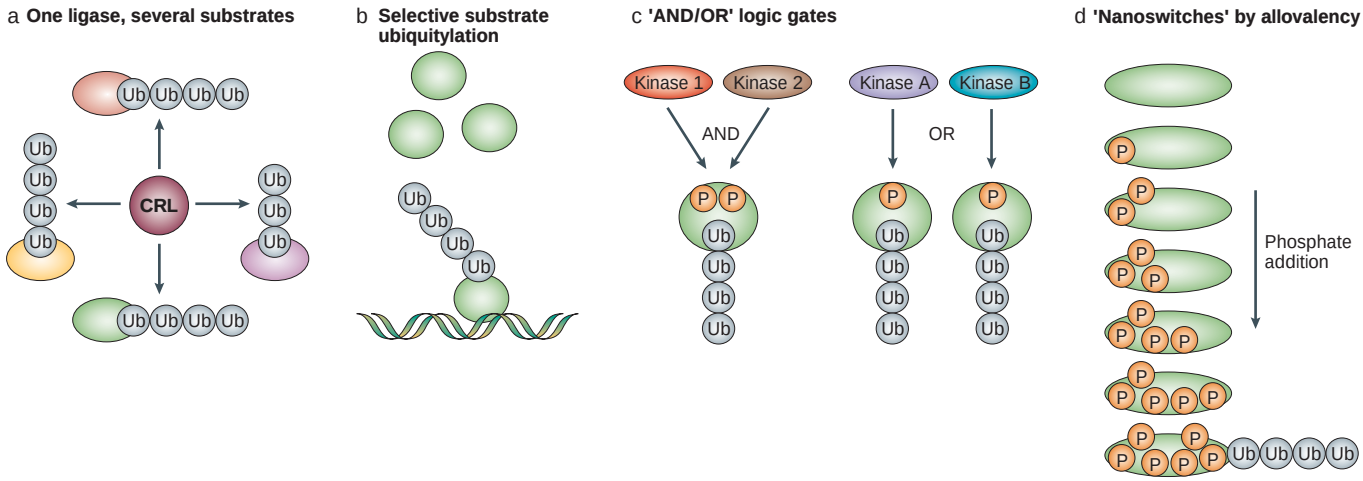

Figure 4 | **Substrate targeting by cullin–RING ligases.** The post-translational modification of substrates that are recognized by cullin–RING ligases (CRLs) influences the timing of their ubiquitylation and subsequent degradation by the 26S proteasome. Several generalized principles are shown. **a** | CRLs might regulate the stability of several substrate proteins in response to similar or distinct signals. **b** | A subpopulation of a CRL substrate (such as a DNA-bound transcription factor) might be ubiquitylated while the bulk pool is unaffected. **c** | The recognition of a substrate by a CRL might require convergent, post-translational-modification signals ('AND' logic gates), or might be sustained by parallel, alternative post-translational-modification pathways ('OR' logic gates). **d** | The ubiquitylation of a substrate might be regulated by the requirement for a threshold level of modification before CRL recognition occurs. P, phosphate; Ub, ubiquitin.

SKP1
(S-phase-kinase-associated protein-1). A 23-kDa protein that functions as an adaptor between CUL1 and F-box proteins. SKP1 was identified as a protein that, together with SKP2, associates with cyclin-A–CDK complexes. It might have other functions, such as binding to centromeres and regulating the assembly of vacuolar ATPases.

RING-H2 DOMAIN
A protein motif that consists of a defined pattern of cysteine and histidine residues (Cys-$X_{(2)}$-Cys-$X_{(9–39)}$-Cys-$X_{(1–3)}$-His-$X_{(2–3)}$-His-$X_{(2)}$-Cys-$X_{(4–48)}$-Cys-$X_{(2)}$-Cys; where X is any amino acid) and that coordinates two zinc molecules. This motif interacts directly with E2 enzymes. Proteins such as MDM2, which have variations of the basic RING motif, can nonetheless sustain ubiquitylation. In CRL RING proteins, the final Cys is an Asp.

BTB DOMAIN
A domain that was identified in 'Broad-complex, Tramtrack and Bric-a-brac' proteins in *D. melanogaster* and that is usually located in the N-terminal region of proteins. Originally known as the POZ domain, this motif is found in several virus proteins. Numerous BTB-domain proteins contain a second protein–protein interaction motif, such as zinc-finger and Kelch motifs.

Generation of 'nanoswitches'. Phosphorylation of Sic1 on CDK sites promotes its binding to SCF^{Cdc4} followed by its ubiquitylation and degradation. Remarkably, though, even though Cdc4 seems to contain one high-affinity phosphopeptide-binding site, Sic1 must be phosphorylated on any six of its nine CDK sites to bind efficiently to Cdc4 (REF. 37). This non-intuitive finding has been rationalized by a mathematical model that shows that the probability of dissociated Sic1 re-binding to Cdc4 before it diffuses away increases exponentially with the number of sites that are phosphorylated[79]. The term 'ALLOVALENCY' has been coined to describe this effect. Provided that Sic1 is phosphorylated distributively *in vivo*, the rate of Sic1 degradation should be proportional to CDK concentration taken to the sixth power (although this remains to be shown). This would result in a 'switch-like' elimination of Sic1 as CDK activity builds during G1 phase (FIG. 4d).

Cullin–RING ligases activate ubiquitin transfer

Despite the importance of CRLs in cell regulation, we know frustratingly little about how they work. In this section, we review what is known about their mechanism of action and highlight the remaining mysteries.

Ubiquitin transfer does not involve a ligase intermediate. The discovery of the RING subunit of SCF sparked a key advance towards understanding how CRLs and the numerous RING-based ligases promote ubiquitylation[7,8,10,26]. Studies on the RING subunit of SCF revealed that it directly binds to and activates the E2 CDC34. Although, by itself, the RING subunit has little effect on CDC34 activity (although see REFS 80,81 for evidence that the RING is sufficient to activate some E2s), the CUL1–RING complex potently activates CDC34 to polymerize free ubiquitin chains[7,8], as well as chains that are attached to *S. cerevisiae* Cdc34 itself[10,26]. Unlike the HECT-DOMAIN ubiquitin ligases, CRLs promote ubiquitylation without forming a covalent intermediate with ubiquitin[10]. The ability of CUL1–RING to activate CDC34 argues that the catalytic core must do other things besides simply bringing CDC34 and substrate into proximity[10].

E2s used by cullin–RING ligases. CRLs can activate isopeptide-bond formation by at least four different E2 enzymes. *S. cerevisiae* Cul1–RING is thought to function exclusively with the E2 Cdc34 *in vivo*, although it can operate with other E2s *in vitro*[24]. Mammalian SCF and other CRLs can work with either CDC34 (UBC3) or E2s of the UBC4/5 FAMILY *in vitro*[82], but it remains uncertain whether this is also true *in vivo*. In addition to CDC34 and UBC4/5, the cullin–RING catalytic core also recruits UBC12, which conjugates the ubiquitin-like protein NEDD8 to cullins[83]. Two aspects of this apparent flexibility in choosing an E2 partner are perplexing. First, CDC34 and UBC4/5 belong to distinct E2 subfamilies[84], which calls into question whether they work and are regulated by identical mechanisms. Second, even though UBC12 is closely related to ubiquitin E2s, it does not transfer NEDD8 to a substrate, but instead it modifies only a single Lys in the cullin subunit, which is only poorly modified (if at all) by the ubiquitin E2s. Perhaps UBC12 docks onto the cullin–RING differently from the ubiquitin E2s.

The mechanism of ubiquitin transfer is unknown. Little is known about how CDC34 or UBC4/5 assemble ubiquitin chains on substrates or how they are switched on

by SCF. The following discussion focuses on the function and regulation of *S. cerevisiae* Cdc34, which has been more intensively investigated. Several lines of evidence point to a role for oligomerization in Cdc34 function. Intragenic complementation data and physical studies indicate that Cdc34 oligomerizes ~~from~~ and that oligomerization involves the unique C-terminal tail[85] and the formation of Cdc34–ubiquitin thioesters, as well as a pair of residues and an acidic loop that are not found in UBC4/5 homologues[86]. Importantly, Cdc34 mutants that cannot oligomerize are inactive *in vivo*[86]. However, it is unclear how Cdc34 oligomers would be accommodated within the 3D architecture of the SCF complex.

A unique feature of ~~CDC34~~ that has drawn considerable attention, is its long C-terminal tail. This C-terminal domain mediates stable binding to SCF (REF. 87). However, a single substitution in the catalytic domain of the E2 Rad6 enables it to restore growth in an *S. cerevisiae cdc34*Δ mutant[88]. So, although it is clearly important, the function that is provided by the unique tail of Cdc4 can be bypassed.

The lack of knowledge regarding how SCF activates the transfer of ubiquitin to a substrate is cast in sharp relief by the detailed 3D structural data that are available for SCF (FIG. 2). The substrate-binding platforms of $SCF^{\beta\text{-TrCP}}$ and SCF^{Cdc4} are situated ~50–60 Å from the predicted location of the E2–ubiquitin-thioester linkage[36,37]. Clearly, a piece of the puzzle about how SCF works is missing. A potential solution to this vexing problem is provided by the 'HIT AND RUN' HYPOTHESIS, which proposes that the activated Cdc34–ubiquitin thioester dissociates from SCF and diffuses until it collides with the Lys residue of a substrate to which it transfers ubiquitin[89]. In support of this proposition, a mutation in *S. cerevisiae* Cdc34 (Phe72Val) that stabilizes its binding to SCF^{Cdc4} greatly diminishes ubiquitylation of the substrate Sic1. However, there are significant challenges facing this hypothesis. For example, once it has been released from SCF, the freely diffusing Cdc34–ubiquitin complex should, on most occasions, diffuse away before it can collide with the substrate. The net effect would be twofold: the nonspecific transfer of ubiquitin to innocent bystander proteins; and the slow, distributive (non-processive) ubiquitylation of substrates that are bound to SCF. By contrast, SCF is exquisitely specific and highly processive.

Flexibility in the selection of substrate lysine residues. Studies with purified components indicate that there can be considerable plasticity in the selection of substrate Lys residues by SCF ubiquitin ligases. This principle is highlighted by Sic1, which has three pairs of closely spaced Lys residues in its N-terminal domain. Any one of these Lys residues is sufficient to sustain the rapid ubiquitylation of Sic1 *in vitro* and the degradation of Sic1 *in vivo*[90]. However, the degradation rates for Sic1 molecules bearing ubiquitin chains at different locations vary by fivefold *in vitro*, which indicates that the context of the ubiquitin chain contributes to the efficacy of substrate degradation by the proteasome. It remains unclear how SCF^{Cdc4}-tethered Sic1 is ubiquitylated efficiently on so many different Lys residues that are distributed over a span of ~50 amino acids. The analysis of other SCF substrates (for example, Gcn4, Far1 and p27) in reconstituted systems indicates that these proteins might also be modified on several Lys residues[38,73,91].

Unlike Sic1, studies on IκB revealed that its ubiquitylation is largely confined to two adjacent Lys residues (Lys21 and Lys22) that are situated nine residues upstream of the first phosphoserine that tethers phosphorylated IκB to β-TrCP. By systematically varying the position of the ubiquitin-acceptor site in a phosphopeptide substrate, Wu *et al.*[36] showed that $SCF^{\beta\text{-TrCP}}$ can efficiently modify a Lys residue that is 10, 14, 18 or 22 residues from a phosphoserine that tethers the peptide to β-TrCP, although a peptide with the Lys situated 10 residues away was ubiquitylated ~1.3–2.5-fold faster than the others. Paradoxically, if this phosphopeptide is a flexible random coil, polymer theory predicts that, on average, the optimal Lys residue would be ~10 Å from the tether point (~40 Å to the thioester bond on the E2) and, at most, the Lys that is situated 10 residues away could only be projected 32 Å towards its target if the peptide and Lys side chain were in a fully extended conformation[36]. The only peptide for which ubiquitylation was strongly compromised (that is, it was more than fivefold slower) had the Lys positioned six amino acids away from the degron — a distance that is far too short to bridge the gap between the E2 and peptide-binding sites. (Parenthetically, this observation provides a further challenge to the hit-and-run model, because it is difficult to explain how moving the Lys acceptor from ten to six residues upstream of the phosphoserine tether would decrease the rate of ubiquitylation by approximately eightfold[36], if, in fact, the transfer of ubiquitin is accomplished by a diffusing E2 molecule that has been released from SCF.) Taken together, the data on SCF^{Cdc4} and $SCF^{\beta\text{-TrCP}}$ indicate that the number of ubiquitylation sites is influenced by the architecture of the ligase complex, the number of independent degrons that tether the substrate to the ligase, and the number and relative position of Lys residues in the vicinity of these ligands. These same factors probably apply to all CRLs.

Regulation of cullin–RING ligases

Whereas post-translational modifications and the diversity of the receptor subunits of CRLs specify which substrates are targeted for ubiquitylation and when ubiquitylation occurs, the activity of the catalytic core is further modulated by several regulatory controls.

Reversible cycles of NEDD8 attachment and removal. All of the cullins evaluated so far[92] can be modified by the covalent attachment of the ubiquitin-like protein NEDD8 to a conserved Lys residue in the cullin-homology domain[93]. NEDDYLATION of CUL1 by UBC12 is not known to require a NEDD8 ligase, but requires the RING subunit[83]. Neddylation enhances CUL1-dependent ubiquitin-ligase activity *in vitro*[94–96], potentially by facilitating the recruitment of ubiquitin-loaded E2s[97]. Genetic studies[93,98–100] indicate that neddylation is important for the functions of CUL1, CUL2 and

F-BOX MOTIF
Originally identified in cyclin F, this structural motif adopts a fold that is similar to the BTB domain, binds to SKP1 and is found in receptors that assemble into CUL1-containing cullin–RING ligases. Proteins that contain the F-box motif are found in all eukaryotes.

AUTOUBIQUITYLATION
This term is loosely used to refer to the covalent transfer of ubiquitins to a Lys of an E2 or E3 component of the ubiquitin-conjugating machinery. This mechanism might regulate the assembly of some cullin–RING ligases by causing the proteasome-mediated degradation of some substrate receptors when substrate levels are depleted.

DEGRON
A portion of a protein that is necessary and sufficient to confer its degradation by the ubiquitin–proteasome system.

LYS-48-LINKED POLYUBIQUITIN CHAIN
Ubiquitin polymers in which the ε-NH_2 group of Lys48 of ubiquitin is linked by an isopeptide bond to the C terminus of the next ubiquitin molecule in the chain. Such a chain can either be unanchored or attached at its proximal (C-terminal) end to the ε-NH2 group of a substrate Lys.

PROTEASOME
A 2-MDa protein complex that degrades ubiquitylated proteins in an ATP-dependent manner.

ALLOVALENCY
A kinetic model that indicates that the affinity observed for some ligand–receptor interactions might increase nonlinearly depending on the polyvalency and flexibility of the ligand. This hypothesis has been proposed for Sic1, as increasing the number of phosphate groups on Sic1 from five to six significantly increases its binding affinity for Cdc4, even though Cdc4 seems to contain only one key phosphopeptide-binding site.

HECT DOMAIN
('homologous to E6-AP C terminus' domain). HECT- and RING-domain-containing proteins represent the two main classes of E3 ubiquitin ligases. In contrast to RING ligases, HECT-domain ligases form an essential thioester intermediate with ubiquitin as it is being transferred from the E2 enzyme to the substrate.

UBC4/5 FAMILY
Two related E2-enzyme families that are structurally distinct from UBC3, the E2 that has been shown, using genetics, to interact with SCF. Although UBC4 and UBC5 ubiquitylate SCF substrates *in vitro*, it is unclear if they do so *in vivo*.

NEDD8
A small protein that is greater than 50% identical to ubiquitin and is conjugated as a single molecule to a specific Lys residue in all cullin-family members. ATP, a heterodimeric E1 (ULA1–UBA3) and the E2 UBC12 are required for the covalent attachment of NEDD8.

'HIT AND RUN' HYPOTHESIS
A mechanistic model for ubiquitin transfer by SCF ubiquitin ligases, which proposes that the dissociation of the E2 enzyme Cdc34 from SCF is required for ubiquitin transfer to the substrate.

NEDDYLATION/DENEDDYLATION
The attachment/removal of NEDD8, in this case, to/from cullin-family members. This cyclical process might regulate the assembly and activity of cullin–RING ligases. It has recently been reported that the RING ligase MDM2 is also regulated by neddylation.

COP9 SIGNALOSOME
(CSN). An eight-subunit complex that was originally identified in plants. It cleaves NEDD8 from cullins and also associates with the deubiquitylating enzyme UBP12.

'JAMM' MOTIF
A metalloprotease motif (His-X-His-$X_{(10)}$-Asp) that was originally identified in the CSN5 subunit of the COP9 signalosome and the RPN11 subunit of 26S proteasome. The JAMM motif is thought to be directly involved in the cleavage of NEDD8 from cullins.

CUL3 *in vivo*, and NEDD8 and its conjugating enzymes are essential in numerous organisms from *Schizosaccharomyces pombe* to mice[93,101], with the notable exception of *S. cerevisiae*[102,103]. *In vivo*, typically only a fraction of the total pool of a cullin protein is neddylated, which indicates that perhaps only a subset of cullin molecules are fully active.

NEDD8 that is conjugated to cullins is detached (a process known as deneddylation) by the COP9 SIGNALOSOME (CSN)[104], and this cleavage is mediated by a 'JAMM' MOTIF in the CSN5 subunit[105] that specifies a metalloprotease-like active site[106,107]. CSN also regulates CRLs by other mechanisms (for a detailed review, see REF. 108) — for example, by recruiting the deubiquitylating enzyme UBP12, which counteracts the intrinsic ubiquitin-polymerizing activity of the catalytic core[109]. In addition, the CSN subunits Csn1 and Csn2 bind to and regulate the activity of *S. pombe* Cul4 by an unknown mechanism[110].

The NEDD8 and ubiquitin isopeptidase activities that are associated with CSN indicate a two-pronged attack on CRL function — deneddylating the cullin decreases the recruitment/activation of E2s[96,97], whereas UBP12 metabolizes ubiquitin chains that are assembled by a bound E2[109]. Paradoxically, genetic studies indicate that CSN is a positive regulator of CRLs, in that loss-of-function mutations in CSN subunits mimic or enhance the detrimental effects of mutations in CRL subunits[99,105,111,112]. So, the dynamic cycling of NEDD8 that is mediated by the opposing activities of UBC12 and the CSN might be required to sustain CRL function (FIG. 5).

Deneddylated cullins are sequestered. CAND1 (also known as TIP120A) was recently identified as a CUL1-interacting protein[113,114]. Although it was originally found in mammalian cells, putative CAND1 orthologues are encoded in the *S. pombe*, *C. elegans*, *A. thaliana* and *D. melanogaster* genomes[113], and a closely-related homologue (TIP120B/CAND2) is found in the human genome. *S. cerevisiae* does not express a CAND1-like protein, which underscores the idea that there are important differences in the regulation of CRLs across species. There is some disagreement about the specificity of CAND1, but it has been reported to bind all cullins except for CUL7, which has not been tested[113–117].

CAND1 competes with SKP1 for binding to CUL1, and only binds to CUL1 molecules that are not conjugated to NEDD8. Moreover, either the conjugation of NEDD8 (REF. 113; although, for a different perspective, see REF. 115), or the simultaneous presence of SKP1 plus high concentrations of ATP[114], can dissociate CAND1 from CUL1. We have therefore proposed that CAND1, the NEDD8-conjugating enzyme UBC12 and NEDD8-isopeptidase CSN sustain SCF activity by promoting cycles of SCF assembly and disassembly[108]. Consistent with this idea, recent data indicate that CAND1 loss-of-function mutants in *A. thaliana* have phenotypes that are reminiscent of SCF mutants[118–120]. Many key questions about the CAND1 cycle remain, including how it operates and how it is regulated.

Pseudosubstrates can regulate cullin–RING function. The identification of heterogeneous nuclear ribonuclear protein U (hnRNP U) as a key binding partner of β-TrCP in mammalian cells pointed to another mechanism of CRL regulation[121]. hnRNP U binds nuclear $SCF^{\beta\text{-TrCP}}$ in competition with phosphorylated IκB (which is a bona fide substrate of $SCF^{\beta\text{-TrCP}}$), albeit with lower affinity. However, unlike phosphorylated IκB, the hnRNP U that is bound to β-TrCP does not become ubiquitylated. hnRNP U might therefore function as an 'affinity gate' that does not interfere with the binding of high affinity substrates such as IκB, but blocks irrelevant proteins from binding nonspecifically to β-TrCP. Alternatively, hnRNP U might function as a shield that blocks the ubiquitylation of β-TrCP in $SCF^{\beta\text{-TrCP}}$ complexes.

RING subunits can contribute to substrate specificity. Metazoans typically express more than one RING protein that can assemble with cullins. In *D. melanogaster*, which expresses three, the SCF^{Slimb}-dependent processing of Ci is blocked in a mutant that lacks the RING subunit Roc1a, whereas the SCF^{Slimb}-dependent turnover of β-catenin (which is known as Armadillo in *D. melanogaster*) proceeds normally[80]. Different RING proteins might therefore specify Armadillo and Ci ubiquitylation even though both substrates use the same receptor subunit. The structure of SCF makes it unlikely that the RING subunit contacts the substrate directly, so the mechanism that underlies this effect remains unclear.

New frontiers in cullin–RING-ligase research

Are there further regulators? A number of factors have been reported that interact with or regulate CRLs, but their functions remain unknown. Among these factors are the mammalian and *S. cerevisiae* Sgt1, *D. melanogaster* Encore and *S. cerevisiae* Cic1 and Cdc48 proteins. Sgt1 binds directly to Skp1, and its activity is required to sustain SCF activity *in vivo* and in *S. cerevisiae* cell lysates[122]. As Sgt1 binds Hsp90 (heat-shock protein of 90 kDa), it might promote the assembly of Skp1-based protein complexes[123,124]. Cdc48 (REF. 125), Cic1 (REF. 126) and Encore[127], on the other hand, bind an SCF component and the proteasome, and are therefore required for the optimal degradation — but not ubiquitylation — of specific SCF substrates.

Formation of ubiquitin-ligase supercomplexes. Some CRLs seem to mix-and-match domains and components from other ubiquitin-ligase pathways. For example, an SCF-like complex identified in neuronal cells of *C. elegans* can use RPM-1 — a large protein with numerous RING domains[128] — instead of ROC-1/RBX-1/HRT-1. This complex, which contains SKP-1, CUL-1 and the F-box-protein FSN-1, might regulate the stability of proteins that are involved in presynaptic differentiation, such as the receptor tyrosine kinase ALK. Further examples of potential combinatorial complexity in the assembly of CRLs are provided by the CUL4-based $DCX^{DET1–COP1}$ ubiquitin ligase[21] and a putative

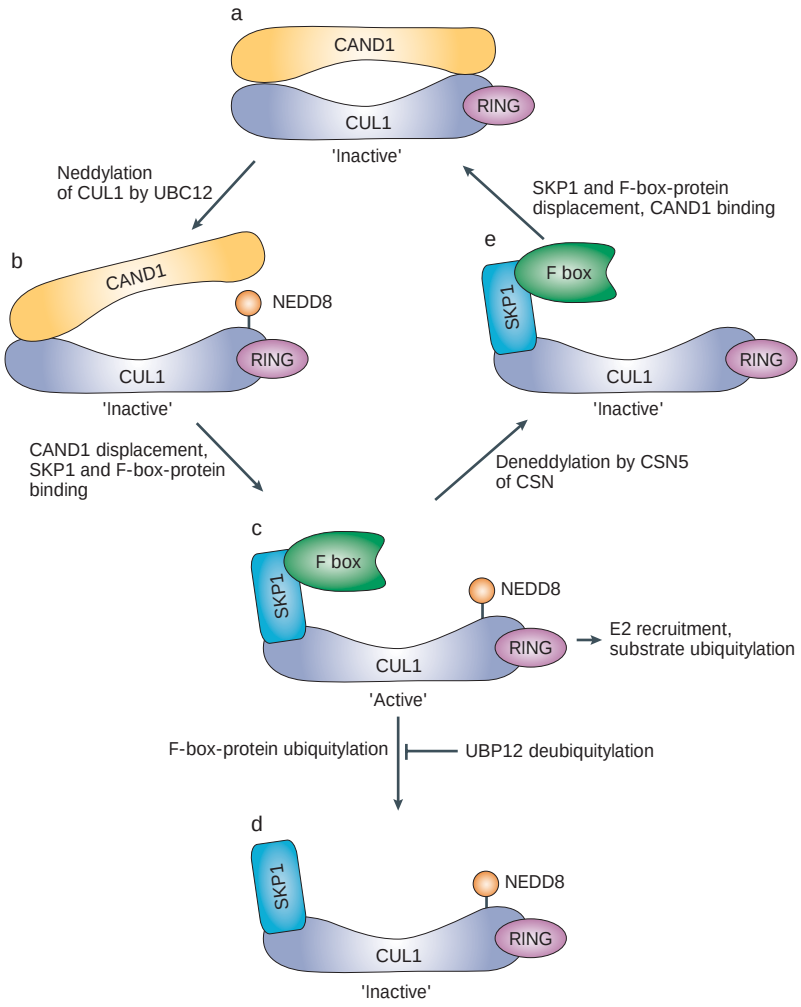

Figure 5 | **The regulation of cullin–RING-ligase activity through the CAND1/NEDD8 cycle.** Cycles of NEDD8 attachment and removal might be important in the regulation of cullin–RING ligase (CRL) activity. **a** | When the enzymatic core of SKP1, CUL1, F-box protein (SCF) is assembled with CAND1, it is held in an inactive state. **b** | The NEDD8 conjugation pathway, which possibly works together with unknown factors, results in CAND1 dissociation. **c** | This might, in turn, enable the assembly of a SKP1–F-box substrate-recognition module on the enzymatic core of SCF. The resulting active complex can ubiquitylate bound substrates. **d** | The interplay between the COP9 signalosome (CSN)-bound deubiquitylating enzyme UBP12 and the ubiquitylation machinery might modulate substrate abundance by regulating F-box-protein stability. Once an F-box protein is ubiquitylated and degraded, a new F-box protein might re-associate with SCF. **e** | Alternatively, NEDD8 might be detached from cullin-1 (CUL1) through the isopeptidase activity of the CSN5 subunit of CSN, which, in turn, might lead to the dissociation of SKP1 and the sequestration of CUL1 by CAND1.

CAND1/TIP120A (cullin-associated and neddylation-dissociated protein-1/TATA-binding-protein interacting protein-120A). A protein that specifically associates with deneddylated cullins to sequester them in an unassembled and inactive state. Putative CAND1 homologues have been identified in most eukaryotic model organisms, except for *Saccharomyces cerevisiae*.

SCF/Parkin ubiquitin-ligase supercomplex[129]. In the former case, as mentioned above, DET1 and COP1 function as a heterodimeric substrate receptor that links the substrate protein Jun to the adaptor protein DDB1, which, in turn, binds CUL4A–RING. Maximal turnover of Jun by this pathway requires the RING subunit of the DCX$^{DET1–COP1}$ complex, but not the RING domain of COP1, even though the RING motif of COP1 has ubiquitin-ligase activity[130].

How is ubiquitylation linked to function? It has recently become clear that the turnover of a given substrate molecule is influenced by its location, assembly and modification state, among other things. For example, the turnover of p27 (REF. 76), cyclin E (REF. 64) and Xic1 (REF. 75) is regulated by their binding to other molecules. More examples of ubiquitylation events that are linked to specific processes such as gene expression and nucleic-acid metabolism are likely to emerge given that many BTB proteins have zinc fingers, and there is even an F-box protein (FBH1) with DNA-helicase activity[131]. Yet another complexity is that different pools of a given protein can be targeted selectively by distinct CRLs. Phosphorylated cyclin E that is bound to CDK2 is targeted by SCF^{CDC4} (REFS 132,133), whereas cyclin E that is not bound to CDK is targeted by SCF^{SKP2} (REF. 134) and a CUL3-based ligase[135]. Likewise, phosphorylated Jun is targeted by SCF^{CDC4} (REF. 136), whereas DCX$^{DET1–COP1}$ can apparently ubiquitylate unphosphorylated Jun[21]. An implication of these findings is that it will be tricky to identify substrates and unravel the role of proteolysis using conventional methods that monitor the entire pool of a protein in a cell or tissue.

Exploiting cullin–RING ligases for research and therapy. CRLs exert enormous control over cellular and organismal physiology, and two general strategies for harnessing this power have been reported. In the first example, an F-box is fused to an interaction partner for a desired target[137,138]. The engineered F-box protein assembles to form an SCF complex that recruits the target protein for ubiquitylation. In an elegant application, a β-TrCP–E-cadherin chimaera recruited cytoplasmic β-catenin (but not the membrane-bound pool) for ubiquitylation and degradation[137]. Unlike conventional genetic methods, this 'protein-knockdown' approach allows the inducible elimination of a specific subset of protein molecules. A second strategy is to construct a bivalent molecule (referred to as a 'protac') that links a desired target protein to a CRL. A protac that comprises the VHL-binding hydroxyproline peptide from HIF1α linked to the androgen dihydroxytestosterone sustains the rapid and efficient turnover of the androgen receptor in cells[139]. It will be interesting to see whether protein-knockdown and protac approaches can be exploited as general tools to modulate protein function.

Concluding remarks

Since the discovery of SCF^{Cdc4} in 1997, ubiquitin ligases that are based on a cullin–RING catalytic core have moved to centre stage in regulatory biology. This superfamily of enzymes controls a broad spectrum of cellular processes by coupling the destruction of regulatory proteins to intracellular and extracellular signals. The specificity of CRLs, which share a modular organization, can be reprogrammed by exchangeable receptor subunits that recruit substrates to the cullin–RING catalytic core. This enables the assembly of potentially hundreds of different ligase complexes. The structural biology of CRLs is providing key insights into the conserved architecture of this superfamily of enzymes and the chemical basis for their specificity, but has also served to highlight how little we know about how these enzymes work. Recent

research has emphasized the importance of a post-translational-modification cycle — neddylation — that might control the dynamic equilibrium of CRL assembly and disassembly. As the field matures, practical applications for harnessing the extraordinary power and specificity of these enzymes to effect cellular regulation are also beginning to emerge. Although progress over the past seven years has been truly impressive, our understanding of the CRL superfamily of enzymes remains in its infancy.

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Acknowledgements

We thank C.-T. Chien, M. Estelle, E. Kipreos, M. Pagano and D. Wolf for their help with the online supplementary information S2 (table). We also thank G. Kleiger for preparing figure 2. M.D.P. is a postdoctoral fellow and R.J.D. is an assistant investigator of the Howard Hughes Medical Institute, which supports their work.

Competing interests statement

The authors declare no competing financial interests.

Online links

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FURTHER INFORMATION

HGNC-Gene-Family Nomenclature — F-Box gene family:
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SUPPLEMENTARY INFORMATION

See online article:
S1 (table) | S2 (table) | S3 (table)
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S1 (table) | **Structures of cullin–RING-ligase subunits, complexes, substrates and regulators**

Structure	Species	Description	PDB accession number	References
SKP1–SKP2	Human	SCF adaptor/receptor	1FQV, 1FS1, 1FS2	1
CUL1–RBX1	Human	SCF enzymatic core	1LDJ	2
CUL1–RBX1–SKP1–F-box (SKP2)	Human	SCF model structure	1LDK	2
APC2 (773–846)	Human	Distant cullin homologue	1LDD	2
Skp1–Cdc4–CPD peptide	Yeast	Substrate recognition by SCF^{Cdc4}	1NEX	3
SKP1–β-TrCP–β-catenin peptide	Human	Substrate recognition by $SCF^{\beta\text{-TRCP}}$	1P22	4
Elongin-BC–VHL	Human	CUL2 complex adaptor/receptor	1VCB	5
Elongin-C–VHL peptide	Yeast	Cul2 complex adaptor/receptor	1HV2	6,7
Hydroxylated HIF1α-peptide–elongin-BC–VHL	Human	Substrate recognition by VHL	1LQB, 1LM8	8,9
PML BTB domain	Human	CUL3 adaptor?	1CS3	10
BCL6 BTB domain	Human	CUL3 adaptor?	1R2B, 1R28, 1R29	11
PLZF BTB domain	Human	CUL3 adaptor?	1BUO	12
CBL–UBCH7	Human	E2 docked on RING E3	1FBV	2
Ubiquitin	Human	Monomer, tetramer chain	1UBQ, 1UBI, 1TBE, 1F9J	13–18
RUB1	*A. thalania*	Monomeric RUB1	1BT0	19
NEDD8	Human	Monomeric NEDD8	1NDD	20
APPBP1–UBA3–NEDD8–ATP	Human	NEDD8-activating enzyme	1R4N, 1R4M	21,22
AfJAMM	*A. fulgidis*	Archaebacterial homologue of Csn5	1R5X, 1O10	23,24

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S2 (table) | **Receptors and substrates of cullin–RING ligases that have been implicated in diverse biological processes**

Organism	Cullin	Receptor	Implicated substrate	Biological Process	References
S. cerevisiae	Cul1	Cdc4	Cdc6	DNA replication	1–3
			Ctf13	Cell cycle	4
			Far1	Cell cycle	5–7
			Gcn4	Transcription	8–10
			Sic1	Cell cycle	11–14
			Cdc4	Autoregulation	15,16
		Grr1	Cln1/2	Cell cycle	13,17,18
			Gic2	Cell polarity	6,19,20
			Grr1	Autoregulation	15,16
		Met30	Met4	Transcription	21–25
			Met30	Autoregulation	24,26
			Swe1	Cell cycle	27,28
		Mdm30	Fzo1	Mitochondrial shape	29
		Ufo1	Ho	Signal transduction	30,31
		?	Cln3	Cell cycle	11,32
		?	Ste7	Signal transduction	33
		?	Clb5	Cell cycle	11
S. pombe	Cul1	Pop1/Pop2	Cdc18	DNA replication	34–36
			Rum1	Cell cycle	34–36
			Cig2	Cell cycle	37
		?	Mid2p	Cell cycle	38
	Cul3	Btb3	Btb3	Autoregulation	39
	Cul4	?	Spd1	DNA replication	40
C. elegans	Cul1	SEL-10	LIN-12	Signal transduction	41
	Cul2	VHL	HIF-1	Transcription	42
		ZIF-1	PIE-1, POS-1, MEX-1/-5/-6	Development	43
		?	CKI-1	Cell cycle	44
	Cul3	MEL-26	MEI-1	Cell cycle	45–49
	Cul4	?	CDT-1	DNA replication	50
D. melanogaster	Cul1	Archipelago	Cyclin E	Cell cycle	51
			dMyc	Transcription	52
		Morgue	Diap1	Apoptosis	53
		Partner of Paired	Paired	Transcription	54,55
		Slimb	Armadillo	Development	56–59
			Cactus	Transcription	60,61
			Cubitus interruptus	Transcription	56,57,59,62

			Dorsal	Transcription	60
			E2F	DNA replication	63
			Period	Circadian-clock regulation	64,65
			Relish	Transcription	66
			Timeless	Circadian-clock regulation	65
	Cul4	?	Cdt1	DNA replication	67
X. laevis	Cul1	β-TrCP	β-catenin	Development	68–71
			Xom	Development	72
		Tome-1	Wee1	Cell cycle	73,74
		?	Xic1	Cell cycle	75–77
A. thaliana	Cul1	COI1	RPD3b	Transcription	78
			Rubisco (small subunit)	Photorespiration	78
		EBF1/EBF2	EIN3	Transcription	79–81
		SLF-S2	S-RNases	Signal transduction	82
		SLY1	RGA	Signal transduction	83,84
		SKP2	E2Fc	Transcription	85
		TLP9	?	Signal transduction	86
		TIR1	AUX/IAA proteins	Transcription	87–89
		UFO	AGAMOUS	Development	90
		ZTL	TOC1	Circadian-clock regulation	91
	Cul3	ETO1	ACS5	Signal transduction	92
H. sapiens/ M. musculus	Cul1	β-TrCP1 and 2 (FBW1, HOS)	ATF4	Transcription	93,94
			β-catenin	Signal transduction	58,95–98
			CDC25a	Cell cycle	99,100
		+ HIV: Vpu	CD4	Viral modulation	101
			EMI1	Cell cycle	102,103
			IκBα	Transcription	60,95,98, 104–111
			NFκB1/p105, NFκB2/p100	Transcription	112–118
			Prolactin receptor	Signal transduction	119
			SMAD3/4	Signal transduction	120,121

			Type-I interferon receptor (IFNAR1)	Signal transduction	122
			WEE1	Cell cycle	123
		CDC4 (FBW7, SEL10)	Cyclin E	Cell cycle	124,125
			Jun	Transcription	126
			Myc	Transcription	127,128
			Notch1/4	Signal transduction	129,130
			Presenilin-1	Regulation	131
		FBS1 (FBX2, NFB42)	Glycoproteins	Protein quality control	132
			Herpes simplex virus type 1: UL9	Viral modulation	133
		FBS2	*N*-glycans	Protein quality control	134
		FBX4	αB-crystallin	Heat-shock protein	135
		SKP2	B-MYB	Transcription	136
		+ Cyclin T1	CDK9	Transcription	137,138
			CDT1	Cell cycle	50,67, 139–141
			Cyclin D	Cell cycle	142–144
			Cyclin E	Cell cycle	145,146
			Papilloma virus: E7	Viral modulation	147
			E2A	Development	148
			E2F1	Transcription	149
			Myc	Transcription	150
			ORC1	DNA replication	151
			p57	Cell cycle	152
		+CKS1	p130	Cell cycle	153,154
		+CKS1	p21	Cell cycle	142,144,155, 156
		+CKS1	p27	Cell cycle	142,145, 157–160
	Cul2	SOCS1/3	IRS1/2	Signal transduction	161
		SOCS1	TEL-JAK2	Cell cycle	162,163
			VAV	Signal transduction	164
		VHL	HIF1α, HIF2α	Transcription	165–175
			RPB7	Transcription	176
			RPB1	Transcription	177
			VDU1/2	Protein quality control	178,179
			hnRNP A2	Transcription	180
			PKCλ	Signal transduction	181

			STRA13	Transcription	182
	Cul3	RhoBTB2	RhoBTB2	Cell cycle	183
		?	TOP1	DNA replication	184
		KEAP1	NRF2	Transcription	185,186
	Cul4	DET1/COP1	Jun	Transcription	187
		Paramyxovirus: V proteins	STAT1/2	Viral modulation	188
		?	CDT1	DNA replication	67
	Cul5	Adenovirus: E1B-55K, E4ORF6	p53	Viral modulation	189
		HIV: Vif	APOBEC3G	Viral modulation	190

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S3 (table) | **Links between pathogens and cullin–RING ligases**

Pathogen: protein	Target	Proposed mechanism of action	References
Agrobacterium: VirF	?	VirF F-box motif links plant protein(s) to SCF	1
Atadenoviruses: RH1/2/4/6	?	Viral F-box proteins link cellular proteins to SCF	2
Human immunodeficiency virus: Vpu	CD4	Vpu promotes degradation of CD4 by linking it to $SCF^{\beta\text{-TrCP}}$	3
Human immunodeficiency virus: Vif	APOBEC3G	Vif SOCS/BC box links APOBEC3G to CUL5–elongin-C	4–6
Faba bean necrotic yellow virus: Clink	RB	F-box and LXCXE motifs of Clink might link RB to SCF	7
Herpes simplex virus type 1: UL9	UL9	UL9 degradation by F-box-protein NFB42 in neuronal cells	8
Human papilloma virus: E7	E7	F-box-protein SKP2 targets E7 for degradation	9
Poxvirus: BTB proteins	?	BTB domain might link cellular targets to CUL3?	10
Adenovirus: E4orf6, E1B55K	p53	E4orf6, E1B55k link p53 to elongin-BC–CUL5–RING	11,12
Paramyxovirus: SV5, HPIV2	STAT1/2	SV5, HPIV2 link STAT1/2 to DDB1–CUL4A	13
SV40: T antigen	CUL7	T antigen sequesters CUL7	14

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